West Nile virus testing ‘the big picture’

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Panel: Stacy Bearden, Steve Schutz and Jerry Davis

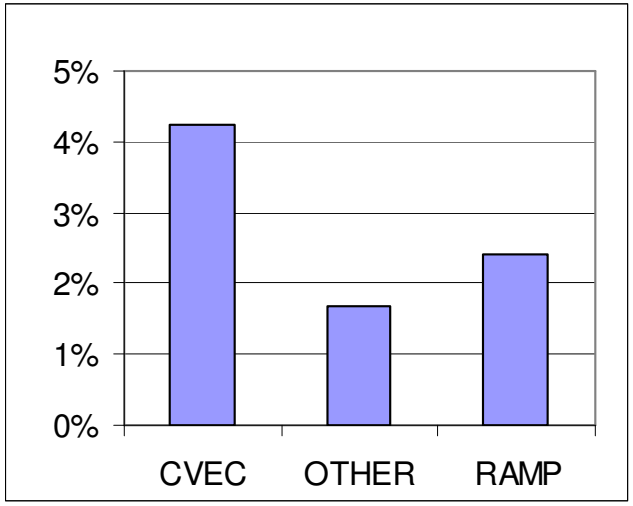
Talk Content

- Review state testing effort
  - Mosquito pools
  - Dead birds
  - Sentinel chickens
- Comparisons among viral assays
  - Proficiency panel results
  - Antigen vs RNA: differences in sensitivity
  - Field comparison: Turlock MAD 2006
  - Impact on time to virus detection and estimates of the MIR
- Is our surveillance program too specific?
  - Review of viruses found in California
  - Likely candidates for importation – where and why?
  - Suggested expansion of surveillance program
- Recommendations

Summary of mosquito, dead bird and sentinel chicken testing by CVEC, VRDL and local agencies

	RT-PCR					
	CVEC			Other		
	WEEV	WNV	Tested	WEEV	WNV	Tested
Mosquito pools	19	665	15,652	0	104	6,210
Dead birds	nd	820	3,453	nd	2	5
	VecTest			RAMP		
	WEEV	WNV	Tested	WEEV	WNV	Tested
Mosquito pools	nd	2	10	nd	89	3,716
Dead birds	nd	469	822	nd	172	290
	VRDL			Other		
	WEEV	Flavi	Tested	WEEV	Flavi	Tested
Sentinel chickens	28	2,189	24,489	0	242	3,114

Percent mosquito pools WNV positive



Based on data downloaded from the Surveillance Gateway, 15 Nov 06

Proficiency panel: CVEC results

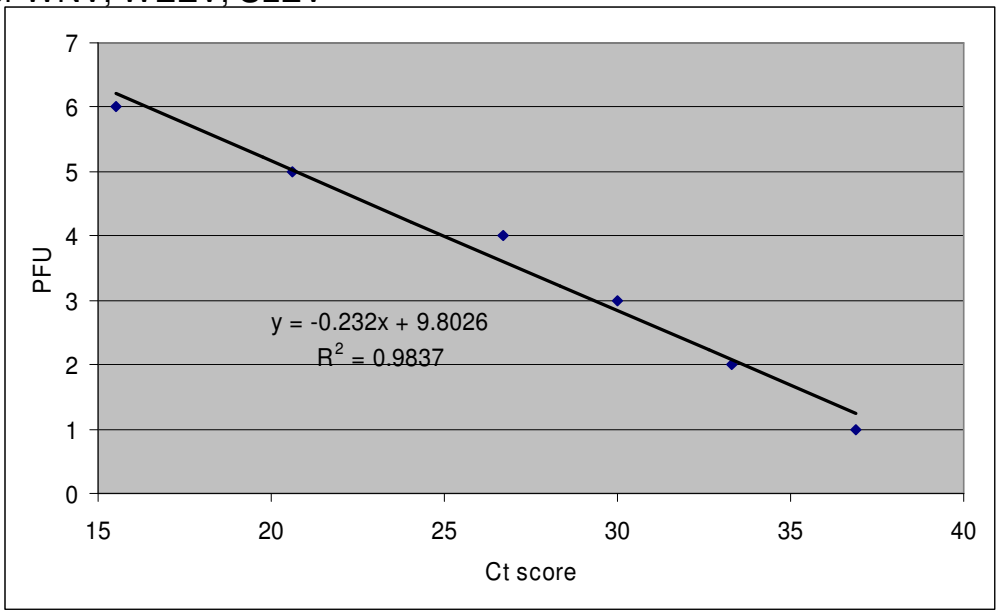
1: CVEC RESULTS	Vial number and Test Results						
Test type	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
VecTest [scored by 2 people]	0	0	0	0	1	2	3
RAMP [units]	0	0	0	27.8	276.2	640	640
RT-PCR [TaqMan Ct values]**	>40	36.9	33.3	30	26.7	20.6	15.5
RT-PCR [TaqMan Ct values]***	>40	>40	34.9	30.2	26.8	21.1	16.12

*Original virus dilution; virus inactivated by 0.5% Triton X-100 overnight incubation in 4^{O}C

**QIAmp viral RNA kit, ABI 7900 TaqMan singlex for WNV

***QIAmp viral RNA kit, ABI 7900 TaqMan multiplex for WNV, WEEV, SLEV

Regression of virus titer [log10 PFU/0.1 ml as a function of real time RT-PCR Ct score



Proficiency panel: local agency results

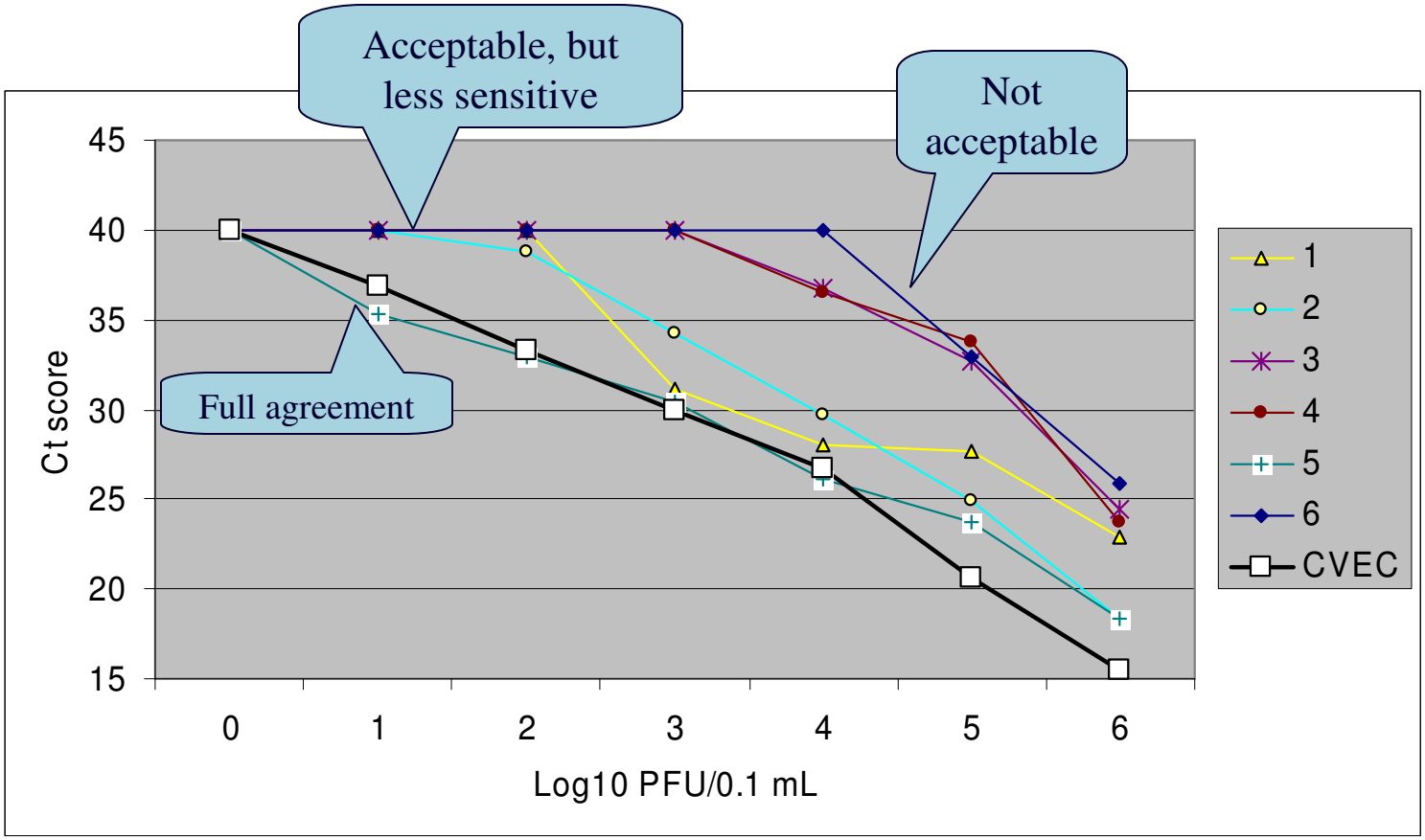
2: VecTest [n = 11 agencies]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	0.0	0.0	0.0	0.0	0.9	2.1	3.0
Max	0.0	0.0	0.5	0.0	2.0	3.0	3.0
Min	0.0	0.0	0.0	0.0	0.5	1.5	3.0

3: RAMP [n = 9 agencies]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	0.0	0.0	1.0	39.6	376.7	640.0	640.0
Max	0.0	0.0	3.2	64.2	640.0	640.0	640.0
Min	0.0	0.0	0.0	18.0	219.6	640.0	640.0

4:RT-PCR [n = 6]	7	1	3	5	6	4	2
log_{10}PFU/0.1 mL*	0	1	2	3	4	5	6
Mean	40.0	39.2	38.6	36.0	32.8	29.3	22.3
Max	40.0	40.0	40.0	40.0	40.0	33.8	25.9
Min	40.0	35.3	32.9	30.4	26.1	23.7	18.4

Problem results

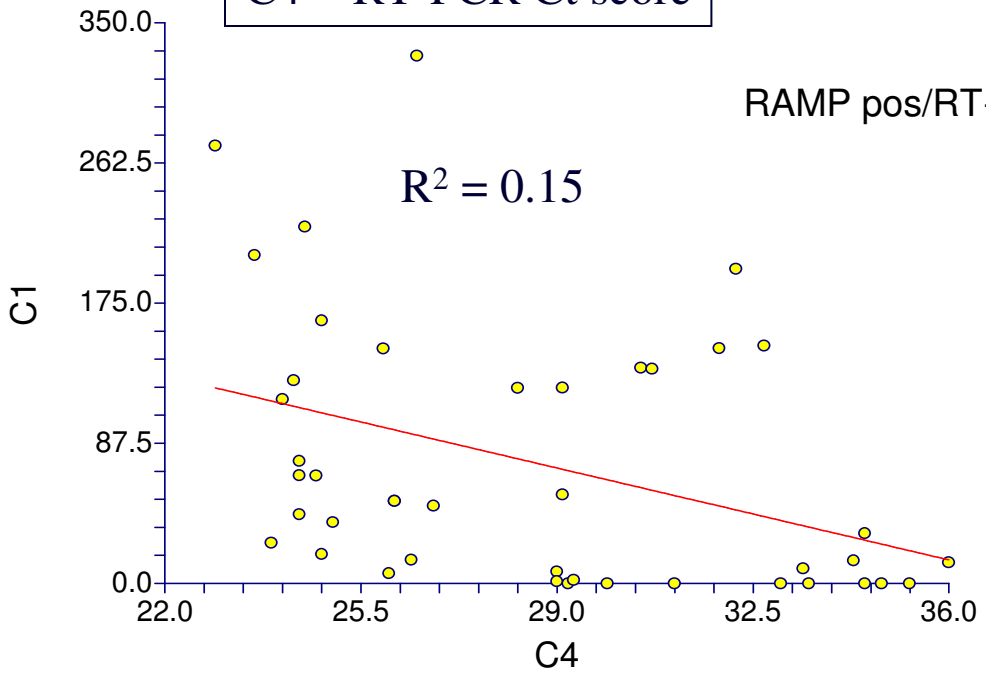
Proficiency panel results: RT-PCR results among local agencies



Operational comparison with Turlock MAD, 2006: all pools tested by RAMP and RT-PCR

	RAMP		
RT-PCR	Pos	Neg	Tot
Pos	26	16	42
Neg	4	996	1000
Tot	30	1012	1042

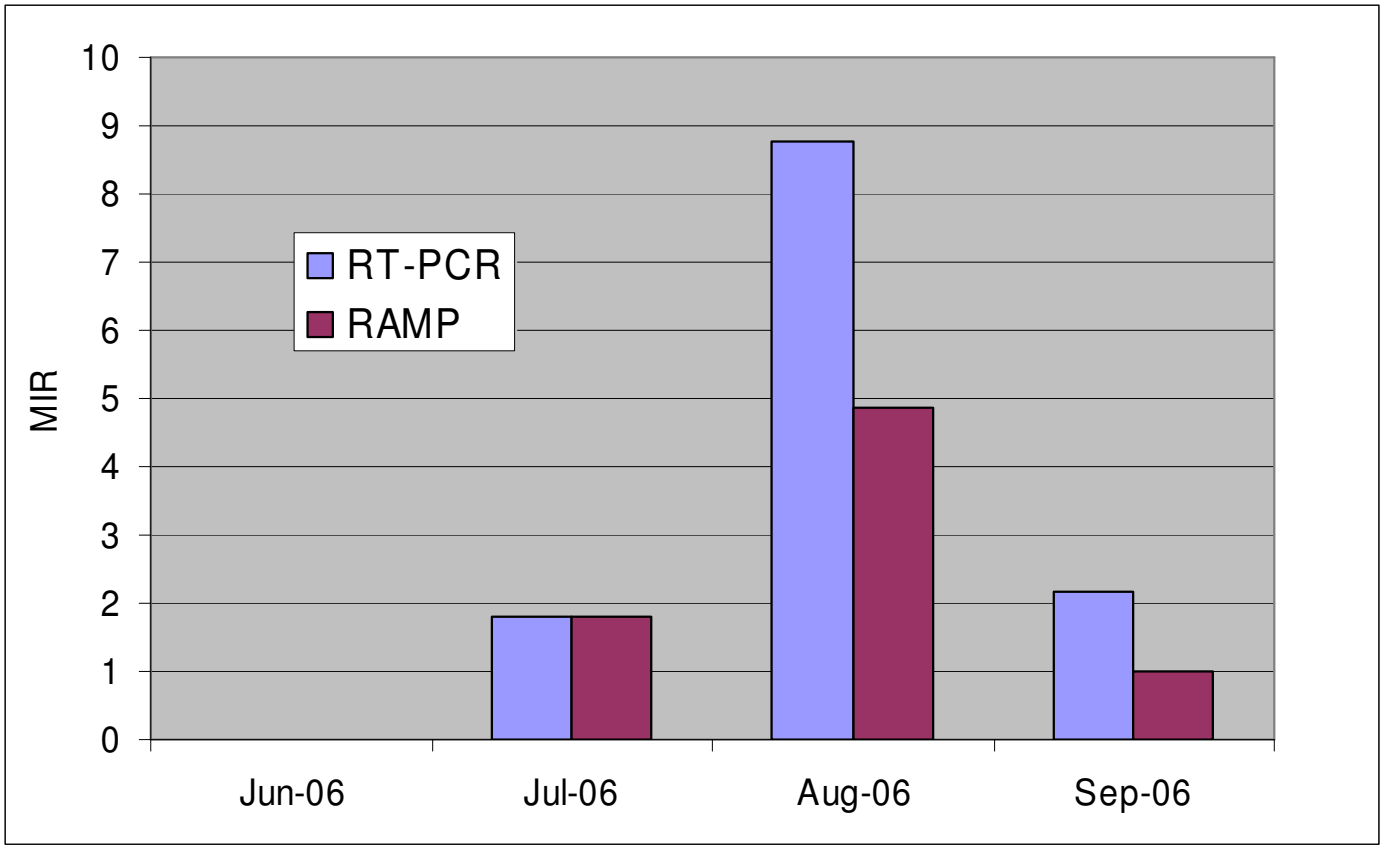
C1 = RAMP score
C4 = RT-PCR Ct score



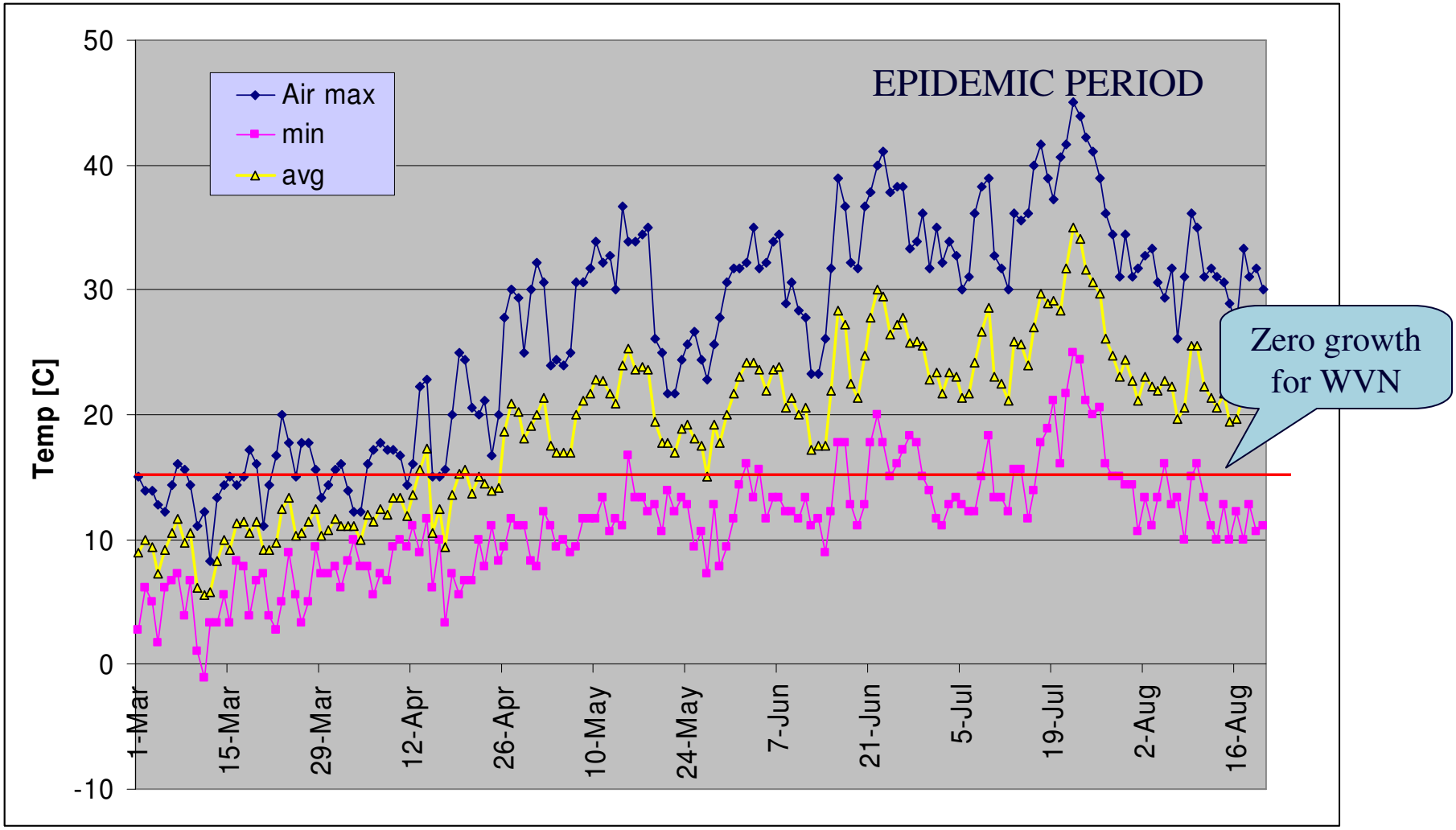
correct ID	Accuracy	0.981
	Specificity	0.996
RAMP pos/RT-PCR positive	Sensitivity	0.619

2006	RAMP	RT-PCR	% Pos
Jun	3	5	60%
Jul	7	7	100%
Aug	10	20	50%
Sep/Oct	6	10	60%
Total	26	42	62%

Change in MIR when pools are tested by RT-PCR vs. RAMP: *Cx. pipiens* data from Turlock MAD, 2006

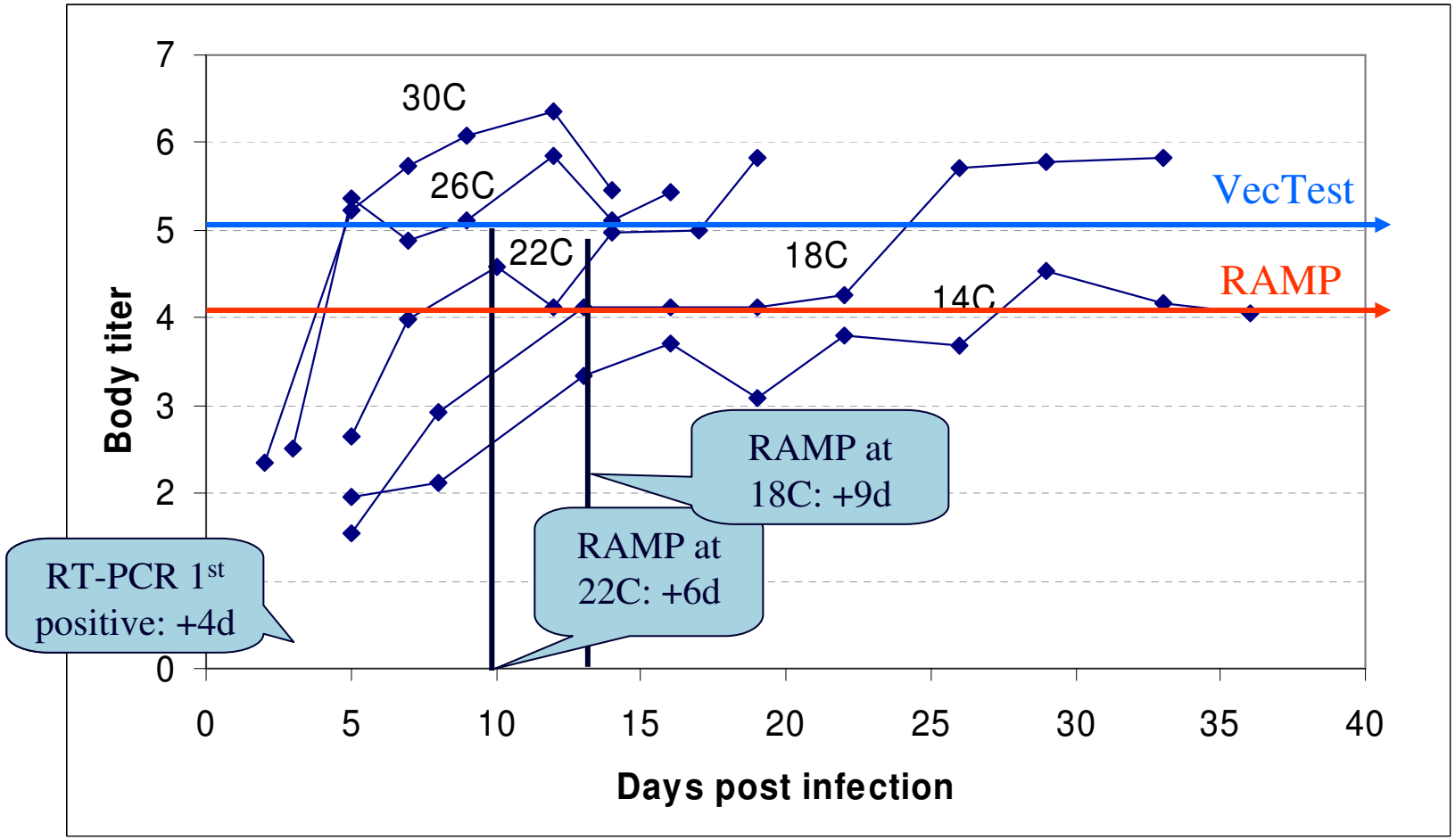


Max-Min and Avg air temperature at Davis, CA, 2006



Impact of sensitivity on time of detection after infection:

Quantity of WNV in $\log_{10}$ PFU in *Cx. tarsalis* females plotted as a function of days when held at 5 temperatures. Shown as horizontal lines are limits for virus detection by VecTest, RAMP and RT-PCR assays [based on current proficiency panels].



Summary

- Proficiency panel results were specific, accurate, and consistent among agencies, but tests varied in sensitivity.
- Thresholds for WNV detection were >100 PFU for multiplex RT-PCR, >10,000 PFU for the RAMP, and >100,000 PFU for the VecTest.
- RT-PCR results were most variable among agencies probably due to differences in chemistry and detection systems.
- Field comparison of RAMP to RT-PCR showed a 38% loss in positive pools
- Decrease in positive pools = decrease in MIR/1,000
- Cool weather slows virus growth within infected mosquitoes and delays detection by less sensitive assays

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Mosquito-borne viruses found in California

Virus	Vector	Reservoir
Alphavirus		
Western equine encephalitis	*Cx. tarsalis, Ae. melanimon*	Birds, rabbits
Flavivirus		
St. Louis encephalitis	*Cx. tarsalis, Cx. quinquefasciatus*	Birds [nestlings?]
West Nile virus	*Cx. tarsalis, Cx. pipiens complex*	Birds [Passeriforms]
Bunyaviruses		
California encephalitis	*Ae. dorsalis* complex	Rabbits
Morro Bay	*Ae. squamiger*	Rabbits?
Jamestown canyon	*Cs. inornata, Aedes*	Rabbits, deer
[Jerry Slough]	*Cs. inornata*	Rabbits?
Northway-like	*Cs. inornata, Anopheles?*	Rabbits
Turlock	*Cx. tarsalis*	Birds
Rhabdovirus		
Hart Park	*Cx. tarsalis*	Birds?
Gray Lodge	*Cx. tarsalis*	??
Orbivirus		
Llano Seco	*Cx. tarsalis*	??

Red = human illness

Some viruses 'on the move' that could invade California

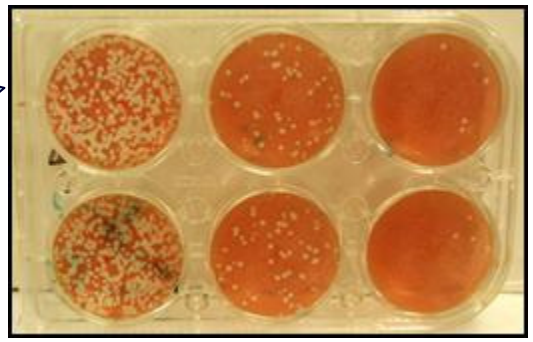
Virus	*Vector*	Reservoir	Distribution
Dengue Fever	*Aedes aegypti, Ae. albopcitus*	Humans, *Aedes* TOT	Asia, Tropical America
Japanese encephaltiis	*Culex tarsalis*	Birds, swine, TOT	Asia
Ross River	*Aedes dorsalis or vexans?*	Humans, *Ae. vigilax* TOT	Australia
Chikungunya	*Aedes aegypti, Ae. albopcitus*	Humans?	Africa, India
Venezuelan equine encephalomyelitis	*Psorophora, Aedes, Culex*	Rodents, Horses	Tropical America
Rift Valley Fever	*Culex, Aedes*	*Aedes* TOT, Humans	Australia

Recommendation: expand surveillance program to capture endemic and new viruses using cell culture

Where will new viruses enter California?

NAFTA trucking

Proposed testing paradigm



Aliquot added to Vero cells at several dilutions to check for CPE and virus detection

RNA extraction and real time RT-PCR with high through put and rapid turn-around-time

Recommendations

- Establish a fixed sampling grid with registered sites at each district to monitor abundance and infection
- Test mosquitoes from permanent grid by RT-PCR to provide early warning and detection of WEEV and SLEV as well as WNV
- Test mosquitoes from points of entry into California using tissue to culture to capture emerging viruses
- Restrict RAMP testing of mosquitoes from ‘roving sites’ to hot summer months to provide quick results for operational decision making
- Restrict RAMP/VecTest testing to American crows